

Development of anti-HMGB1 antibody drug

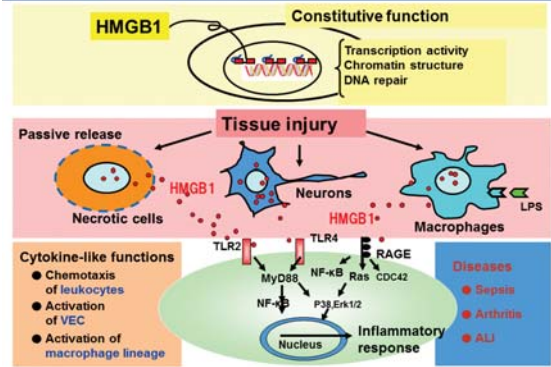
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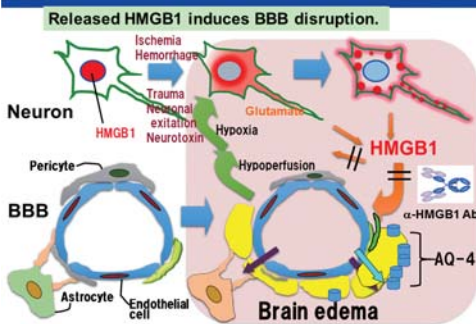
ABSTRACT

High mobility group box-1 (HMGB1) plays an important role in many kinds of inflammatory diseases. Anti-HMGB1 monoclonal antibody therapy has been demonstrated to be beneficial for the treatment of brain injuries induced by ischemia, hemorrhage and trauma. Moreover, anti-HMGB1 suppressed epileptogenesis and neuropathic pain. We developed humanized monoclonal antibody that neutralizes the activity of HMGB1. This antibody will be applicable for many kinds of diseased listed above.

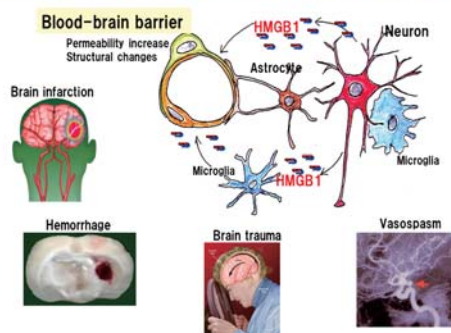
HMGB1 is released from different cells as DAMP



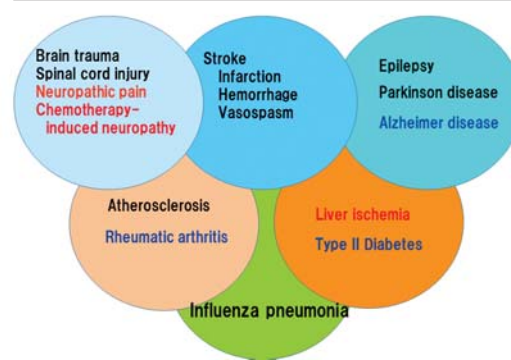
HMGB1 release and disruption of BBB



HMGB1 may be a mediator of Neurovascular unit



Anti-HMGB1 monoclonal Ab applicable for many diseases



2009-2011 Grant from Ministry of Health, Welfare and Labour "Anti-HMGB1 antibody therapy" PI: Dr. Masahiro Nishibori

High Mobility Group Box-1 (HMGB1) as a novel target for drug development

HMGB1 domain structure: A-box, B-box, C-terminal epitope, C-Tail.

Receptors: TLR2, TLR4, RAGE, MyD88, NF-κB, P38, Erk1/2.

Brain Inflammation: BBB disruption, Microglia activation, Vasospasm.

Brain trauma (Fluid percussion): Evans blue leakage (6h), T2-WMR (12h).

Spinal cord injury (Th10): Paralysis of hind paw (8W), Red: Microglia (Green: Astrocyte), Albumin leakage (8W).

Target validation: Rat mAb (#10-22) → Patent 2013-013602, PCT/JP2013/82860, Humanized anti-HMGB1 mAb.

Patent search & examination: No conflict & invasion (April, 2015).

Awards: Japan Invention Award 2009, Bioshiness Japan Award 2012, JST International support.

Brain and spinal cord injuries due to biological responses: Regulation by anti-HMGB1

Brain trauma (Fluid percussion): Control mAb vs Anti-HMGB1 mAb. Shows reduced Evans blue leakage and T2-WMR signal.

Spinal cord injury (Th10): Control mAb vs Anti-HMGB1 mAb. Shows reduced paralysis of hind paw and albumin leakage.

Vasospasm: Microglia activation, Inflammation.

HMGB1 score graph: Shows significant reduction in HMGB1 score over 56 days for the anti-HMGB1 mAb group.

Pain regulation by a novel method different from opioids

Resistant to opioids & nerve block: Hyperalgesia due to peripheral nerve injuries, Hyperalgesia due to CNS injuries, Hyperalgesia due to drugs and radiation therapy.

PSNL model (Dr. Nakata & Morioka, PLoS ONE 2013): Shows pain relief with anti-HMGB1 mAb.

Central Mechanism: Spinal cord, Primary sensory N (DRG), Secondary sensory N, Activated microglia.

Peripheral mechanism: Macrophage, RAGE, Ca²⁺, Sensory nerve, Permeability increase.

Anti-tumors: Pacitaxel, Vincristine, Macrophage lineage.

Non-GMP (#10-22) toxicity test: a single injection (10 mg/kg, i.v.)

Histological exams: 24 organs. Control vs Anti-HMGB1 #10-22.

Non-GMP (#10-22) toxicity test: Repeated administration (0.75-3.0 mg/kg, 6d). Blood cells, No subacute toxicity.

Physiological chemical analysis: Total proteins, Albumin, AST, BUN, Creatinine, Glucose. Control vs Anti-HMGB1.

Japanese monkey: Demonstration of HMGB1 translocation in primate. HE, Red: HMGB1, Green: HMGB1 antibody.

Development of original humanized antibody recognizing C-terminal end of HMGB1

Humanization of the variable region of lambda chain: Rat monoclonal antibody (#10-22) → Human antibody frame (Conservation of CDR) → CHO-K1 expression system → Purification of Ab.

Humanization of the variable region of lambda chain: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Sequence alignment: Shows high identity between rat and human lambda chain variable regions.

EV007156 binds all types of HMGB1 (OKY-001)

Immunoblotting: Necrotic, Apoptotic, Activated, Secondary Necrotic.

EV007156: 25kDa, S6, G4, 25kDa.

S6 and G4 are anti-HMGB1 human Abs from MedImmune, and were produced by Evvec, Inc. for direct comparison.